-- (new) 62. A recombinant adenoviral vector wherein said vector comprises at least a lethal deletion or mutation in two gene regions selected from the group consisting of E1, E2A, E4 early gene regions, viral structural genes, and additionally comprises a transgene under the control of the human phosphoglycerate kinase promoter, so that when rescued the resulting recombinant adenovirus required for replication complementation of both the E1 and E4 adenoviral early gene regions.

(new) 63. A DNA plasmid comprising an inducible promoter operably linked to nucleotide sequences encoding cytotoxic gene products of adenoviral E4 and E2A gene regions.

(new) 64. The DNA plasmid of Claim 37 which further expresses an adenoviral E2A gene fragment operably linked to an inducible promoter.--

REMARKS

The claims have been amended as suggested by the Examiner to more particularly point out and distinctly claim the invention. The specification has been amended to add ATCC deposit numbers in order to correct any informalities and does not represent new matter. New Claims 62 to 64 are fully supported by the specification and do not represent new matter. Claims 43-48 and 51-61 have been canceled in the present application without prejudice to prosecuting these claims in related applications. The cancellation of Claims

43-48 and 51-61 obviates all of the Examiner's rejections in view of these claims.

1. The Invention

The present invention relates to novel recombinant adenoviruses characterized by at least two lethal deletions in early gene regions and the novel packaging cell lines that function to propagate these replication deficient adenoviruses. The deletion of two essential regions, both the E1 and E4 regions, dramatically minimizes or eliminates the pathogenic effects of direct cytotoxicity to the targeted cells and inflammatory responses in the human body. The resulting virus, however, is replication defective and requires the E1 and E4 functions *in trans* in order to replicate. However, since the expression of E1 activates the expression of E4 which is cytotoxic, no one has been able to develop a cell line that expresses and provides these functions to support viral replication and packaging.

The present invention provides a novel packaging cell line which complements functions of E1 and E4, and optionally the E3 DNA regions. The present invention overcomes the difficulty of establishing a cell line to complement the E1 and E4 functions deleted from the recombinant adenoviruses of the present invention by providing a 293 host cell which contains the E1a, E1b, E2a and E4 gene regions. The E4 ORF6 gene region has been introduced into 293 cells and placed under the control of an inducible promoter, *e.g.*, a CREB or a tetracycline inducible promoter, so that in the uninduced state, expression is low enough to avoid toxicity to the host

cell, but in the presence of tetracycline is sufficiently activated to make enough E4 ORF6 gene product to complement the E4 deleted region during virus production.

2. The Rejections Under 35 U.S.C. §112 Should Be Withdrawn

The specification is objected to and Claims 53 and 54 are rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. Claims 53 and 54 have been canceled, thus obviating the Examiner's rejection under 35 U.S.C. §112. Applicants submit that these rejections are in error and should be withdrawn for the reasons explained below.

Claims 49 to 52 are rejected under 35 U.S.C. § 112, first paragraph, based on the contention that the specification while being enabled for 293 derived packaging cell lines capable of supporting growth of the claimed mutant adenovirus vectors, does not enable non-293 derived cell lines. Applicants do not agree that the specification does not enable packaging cell lines other than those derived from 293 cells. However, in order to expedite prosecution of the pending claims, Applicants have amended the claims to recite packaging cell lines as derived from 293 cells, without prejudice. Applicants reserve the right to prosecute claims to packaging cell lines other than those derived from 293 cells in related applications. Thus, the amendment to the claims obviates the Examiner's rejection of the Claims 49-52 under 35 U.S.C. § 112, first paragraph.

Therefore, the rejections under 35 U.S.C. §112, first paragraph are obviated in view of the amendments to the claims and thus, should be withdrawn.

3. The Rejections Under 35 U.S.C. §102 Should Be Withdrawn

Claims 49 and 50 drawn to a packaging cell line that supports the growth of a mutant adenovirus containing at least two lethal deletions in the E1, E2A and E4-ORF6 early gene regions are rejected under 35 U.S.C. §102(b) as being anticipated by Weinberg et al. These claims have been amended to recite packaging cell lines that support the growth of recombinant adenoviral vectors that contain at least two lethal deletions, at least two lethal mutations or at least one lethal mutation and one lethal deletion selected from the group of E1, E2a and E4 early gene regions.

The legal test for anticipation under 35 U.S.C. §102 requires that each and every element of the claimed invention be disclosed in a prior art reference in a manner sufficient to enable one skilled in the art to reduce the invention to practice, thus placing the public in possession of the invention. W.L. Gore Associates v. Galock, Inc., 721 F.2d 1540, 1554 (Fed. Cir. 1983) cert. denied 469 U.S. 851 (1984); In re Donohue, 766 F.2d 531 (Fed. Cir. 1985). Anticipation under U.S.C. §102 requires identify of invention. Scripps Clinic & Research Fdn. v. Genentech Inc., 927 F.2d 1565 (Fed. Cir. 1991).

The reference cited by the Examiner, Weinberg, describes the characterization of 293 cells. 293 cells will support the

growth of adenoviruses containing deletions in the E1 region and non-essential deletions in the E4 region. Since ORF6 is the essential region of the E4 early gene region, a deletion in the E4 early gene region -- except for ORF6 -- does not constitute a lethal deletion or mutation. In addition, 293 cells could not support the growth of a replication defective adenovirus containing a lethal detection in the E4 early gene region, and thus are clearly not encompassed by the invention as claimed. Thus, the claims as amended are not anticipated by the prior art and the Examiner's rejections under 35 U.S.C. § 102 should be withdrawn.

4. The Rejections Under 35 U.S.C. §103 Should Be Withdrawn

Claims 37-40 and 42 drawn to a DNA plasmid comprising an inducible promoter linked to nucleotide sequences encoding an adenoviral gene fragment E4 ORF6 are rejected under 35 U.S.C. §103 as obvious over Ketner et al. in view of Jyan-Gwo et al. Claim 41 drawn to a DNA plasmid comprising a tetracycline responsive promoter linked to nucleotide sequences encoding an adenoviral gene fragment E4 ORF6 is rejected under 35 U.S.C. §103 as obvious over Ketner in view of Jyan-Gwo and Gossen.

The Examiner contends that Ketner describes plasmids comprising a promoter linked to the adenoviral E4 early region, and that Jyan-Gwo and Gossen teach that α-inhibin and tetracycline responsive promoters are inducible promoters. The Examiner concludes that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the promoters described in Jyan-Gwo and Gossen and

link them to the nucleotide sequences described in Ketner which encode the E4, to arrive at the present invention. This rejection is in error for the reasons explained below.

A finding of obviousness requires a determination of the scope and content of the prior art, the level of ordinary skill in the art, the differences between the claimed subject matter and the prior art, and whether the differences are such that the subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. Graham v. Deere 383 U.S. 1 (1996). The proper inquiry is whether the art suggests the invention, and whether the art provides one of ordinary skill in the art with a reasonable expectation of success. In re O'Farrell 853 F.2d 894, 7 USPQ2d 1673 (Fed. Cir. 1988). Both the suggestion and the reasonable expectation of success must be founded in the prior art and not in the Applicants' disclosure. In re Vaeck 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

In the present instance, the relevant inquiry is, first, whether the art relied on by the Examiner suggests the use of inducible promoters, such as cAMP-responsive promoters and tetracycline responsive promoters, to regulate expression of a cytotoxic adenoviral gene region. Moreover, assuming arguendo that the prior art provided such a suggestion, the second inquiry is whether it provides one of ordinary skill in the art with a reasonable expectation of success, i.e., that inducible promoters, such as the cAMP-responsive promoters or tetracycline-responsive promoters, are stringent enough to regulate expression of a cytotoxic adenoviral gene in a packaging cell, so as not to kill the cell. In re O'Farrell;

In re Vaeck, supra. Applicants assert that the prior art neither suggests the plasmids of the present invention, nor gives any reasonable expectation of success.

The Examiner's rejection is based on several erroneous interpretations of the art cited. First, the Examiner contends that a person of ordinary skill in the art would recognize that the choice of an inducible promoter to drive expression of the E4 ORF6 would simply be optimization of the process parameters of the system described in Ketner. There is no suggestion in Ketner to choose an inducible promoter to drive expression of the E4 ORF6. Ketner describes plasmids containing various E4 ORF deletions all of which are under the control of an intact E4 promoter. Ketner uses these plasmids to transiently transfect 293 cells to achieve packaging of adenoviral E4 mutants. Ketner observes that low levels of packaging are achieved, and attributes this to "low efficiency of transfection, insufficient expression of E4 products from plasmids, low E4 gene dosage due to failure of the plasmid sequences to replicate with the viral genome, or a combination of those factors" (Ketner, page 3045, lines 20 to 22). Ketner recognizes that there is poor transfection efficiency and low levels of expression of E4, but Ketner does not suggest that the solution to this problem is an inducible promoter.

One of ordinary skill in the art may have recognized that in order to optimize the process parameters described in Ketner, one should improve transfection protocols or switch the E4 promoter for a stronger promoter in order to improve levels of E4 expression -- but why would one of ordinary skill in the art choose an inducible promoter to improve levels of

E4 ORF6 expression given the unimpressive levels of induction reported in the prior art for inducible promoters, (e.g., stimulation of the α-inhibin promoter only results in three fold induction in promoter activity (Jyan-Gwo). Thus, there is simply no motivation in the prior art to use an inducible promoter to improve the levels of E4 ORF6 expression.

Further, one of ordinary skill in the art would not view an inducible promoter as an improvement of the process parameters of the system described in Ketner. Jyan-Gwo describes the characterization of a cAMP-responsive promoter and Gossen describes a tetracycline-responsive promoter to regulate expression of a reporter gene, firefly luciferase. Ketner recognizes that there is a need to achieve higher levels of expression of E4 in a transient transfection assay -- not that there is a need to achieve tightly regulated expression of E4 ORF6. Thus, one of ordinary skill in the art would select a stronger promoter, not an inducible promoter. Thus, there is simply no motivation to combine Jyan-Gwo and Gossen with Ketner.

Moreover, assuming arguendo that there was a suggestion in Ketner, or any other prior art reference, to use an inducible promoter to tightly regulate the expression of E4 due to its toxicity, there is no expectation of successfully using a cAMP-responsive promoter to achieve this goal, thus Claims 38 to 40 are separately patentable for additional reasons. The Examiner states that one would have been motivated to use the promoters of Jyan-Gwo since it was well known at the time that promoters containing cAMP-responsive elements inducibly regulate gene expression. However, there

were many inducible promoters known at the time. Therefore, the suggestion lacking in Ketner is certainly not provided by Jyan-Gwo. Moreover, the prior art provides no expectation that cAMP-responsive promoters work sufficiently to control the toxicity of E4 gene products. One skilled in the art would choose an inducible promoter that when uninduced resulted in little or no expression of the toxic gene, so as not to kill the producer cell. In contrast to this type of inducible promoter, the invention describes cAMP-responsive promoters--i.e., promoters which are inducible by cAMP, but which maintain a basal level of constitutive expression as reported by Jyan-Gwo, the secondary reference relied on by the Examiner (e.g., see Jyan-Gwo at pp. 298-299). Further, the cAMP-responsive promoter described in Jyan-Gwo only resulted in a three fold increase in reporter gene activity following induction, which is not a very impressive level of induction of promoter activity. Thus, one skilled in the art would not have expected a cAMP-responsive promoter to work sufficiently to control the toxicity of the E4 region gene products in a producer cell.

In view of the foregoing, the art relied on by the Examiner does not render obvious the replication-defective adenoviruses and packaging cell lines of the claimed invention.

CONCLUSION

Applicants respectfully request entry and consideration of the foregoing amendments and remarks. The claims are believed to be free of the art, and patentable. Withdrawal of

all the rejections and objections and an early allowance is earnestly sought.

Respectfully submitted,

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Laura A. Coruzzi (Reg. No.)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosure